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UNIVERSIDADE Ð
COIMBRA

Ariana Maria Figueiredo Saraiva

QUALITY AND SAFETY OF EDIBLE SEAWEEDS FROM THE PORTUGUESE COAST

Dissertação no âmbito do Mestrado em Segurança Alimentar orientada pelo Professor Doutor Fernando Jorge dos Ramos e co-orientada pela Doutora Amparo Celeste Gonçalves e apresentada à Faculdade de Farmácia da Universidade de Coimbra.

Setembro de 2019

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Dissertação de Mestrado em Segurança Alimentar, sob orientação do Professor Doutor Fernando Ramos e da Investigadora Auxiliar da Divisão de Aquacultura e Valorização do Instituto Português do Mar e da Atmosfera, Doutora Amparo Gonçalves, apresentada à Faculdade de Farmácia da Universidade de Coimbra, cujo trabalho analítico foi realizado no Instituto Português do Mar e da Atmosfera.

Coimbra, Setembro de 2019

O trabalho apresentado nesta dissertação foi realizado no Instituto Português do Mar e da Atmosfera, sob a orientação do Professor Doutor Fernando Ramos e da Doutora Amparo Gonçalves, no âmbito do Mestrado em Segurança Alimentar da Faculdade de Farmácia da Universidade de Coimbra.

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Abstract

Seaweeds are abundant, although their incorporation in human diet is poorly exploited in the Western World. However, in a near future a stronger focus in their utilization is expected, as a food commodity, to meet the challenge of finding new food and sustainable resources with a smaller carbon footprint. Thus, the present study aimed to characterise the nutritional profile of *Undaria pinnatifida* (Harvey) Suringar, *Porphyra linearis* (Linnaeus) Kützing, and *Porphyra umbilicalis* (Greville), three seaweeds that commonly occur in the Portuguese coast, to evaluate their potential for human consumption.

Specifically, the contents of moisture, ash, protein and fat, as well as the fatty acids profile were determined. An elemental analysis was conducted to quantify cooper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), selenium (Se), calcium (Ca) and zinc (Zn). The contents of potential toxic elements such as cadmium (Cd), lead (Pb), mercury (Hg), and total arsenic (As) were also determined. All the analyses were carried out in freeze dried samples according to reference methods. Overall, significant differences were found between *U. pinnatifida* and both species of the genus *Porphyra*. The former species presented the highest levels of Na, K and Mg (29.3±3.2, 48.8±3.4 and 9.7±0.1 g/kg DW, respectively) and the lowest contents of Mn and Cu (8.3±0.4 and 1.0±0.3 mg/kg DW, respectively). The content of Zn differed significantly among the three species, ranging from 17.6±0.6 (*U. pinnatifida*) to 61.1±0.1 mg/kg DW (*P. linearis*). The highest nutritional contributions regarding the dietary reference values were found for Ca (7.37% in *P. umbilicalis*), Mg (12.99% for men and 15.15% for women in *U. pinnatifida*), Fe (5.84% for men and 4.02% for women in *P. umbilicalis*) and Mn (5.56% in *P. umbilicalis*). Regarding toxic elements, the concentration of total As ranged from 46.7±0.7 to 54.9±0.4 mg/kg DW while the highest Cd content (1.24±0.05 mg/kg DW) was reported for *P. linearis*. The Pb and total Hg levels were below the European limits. Regarding the macronutrients profile, it is worth mentioning the high protein content of all the samples analysed, as well as an adequate n-3/n-6 PUFA ratio. Further studies on contaminants of marine seaweeds are required since they have a high absorption capacity.

Keywords: elemental composition; fatty acids; macroalgae as food; *Porphyra* spp.; toxicity; *Undaria pinnatifida*.

Resumo

As algas são abundantes, embora a sua incorporação na dieta humana seja pouco explorada no Ocidente. No entanto, num futuro próximo, espera-se um foco mais forte na sua utilização, como produto alimentar, para enfrentar o desafio de encontrar novos alimentos e recursos sustentáveis com uma menor pegada de carbono. Assim, o presente estudo teve como objetivo caracterizar o perfil nutricional de *Undaria pinnatifida* (Harvey) Suringar, *Porphyra linearis* (Linnaeus) Kützing e *Porphyra umbilicalis* (Greville), três algas marinhas que ocorrem comumente na costa portuguesa, para avaliar o seu potencial para consumo humano.

Especificamente, os teores de humidade, cinzas, proteínas e gorduras, bem como o perfil de ácidos gordos foram determinados. Uma análise elementar foi realizada para quantificar cobre (Cu), ferro (Fe), potássio (K), magnésio (Mg), manganês (Mn), sódio (Na), fósforo (P), selénio (Se), cálcio (Ca) e zinco (Zn). Os teores de potenciais elementos tóxicos tais como cádmio (Cd), chumbo (Pb), mercúrio (Hg) e arsénio (As) total também foram determinados. Todas as análises foram realizadas em amostras liofilizadas de acordo com métodos de referência. Em geral, foram encontradas diferenças significativas entre *U. pinnatifida* e as duas espécies do género *Porphyra*. As espécies anteriores apresentaram os maiores teores de Na, K e Mg (29,3 ± 3,2, 48,8 ± 3,4 e 9,7 ± 0,1 g / kg DW, respectivamente) e os menores teores de Mn e Cu (8,3 ± 0,4 e 1,0 ± 0,3 mg / kg DW, respectivamente). O teor de Zn diferiu significativamente entre as três espécies, variando de 17,6 ± 0,6 (*U. pinnatifida*) a 61,1 ± 0,1 mg / kg de DW (*P. linearis*). As maiores contribuições nutricionais foram encontradas para Ca (7,37% em *P. umbilicalis*), Mg (12,99% para homens e 15,15% para mulheres em *U. pinnatifida*), Fe (5,84% para homens e 4,02% para mulheres em *P. umbilicalis*) e Mn (5,56% em *P. umbilicalis*). Em relação aos elementos tóxicos, a concentração de As total variou de 46,7 ± 0,7 a 54,9 ± 0,4 mg / kg DW, enquanto o maior teor de Cd (1,24 ± 0,05 mg / kg DW) foi reportado para *P. linearis*. Os níveis de Pb e Hg total ficaram abaixo dos limites europeus. No que se refere ao perfil de macronutrientes, deve-se destacar o alto teor de proteína de todas as amostras analisadas, bem como uma relação PUFA n-3/n-6 adequada. Estudos adicionais sobre contaminantes de algas marinhas são necessários, uma vez que estas possuem uma alta capacidade de absorção.

Palavras-chave: ácidos gordos; composição elementar; macroalgas como alimento; *Porphyra* spp.; toxicidade; *Undaria pinnatifida.*

1. Introduction

Seaweeds, or macroalgae, live in costal and marine habitats in all climatic regions around the world. Historically, seaweed been used as human foodstuff by our ancestors who evolved by foraging in coastal regions (Mouritsen, 2013; Pérez-Lloréns *et al.*, 2018). Seaweeds may have played a role not only for human nutrition and health (Cornish et al., 2015) but also been fundamental for human brain evolution (Cornish *et al.*, 2017) since they contain essential elements for brain development, specifically polyunsaturated omega-3 and omega-6 fatty acids, taurine, magnesium, zinc, vitamin B12, and iodine (MacArtain *et al.*, 2007; Pereira, 2011; Holdt and Kraan, 2011; Brown *et al.*, 2014).

Macroalgae are multicellular, fast-growing, macroscopic nonvascular organisms that contain chlorophyll, with sizes reaching up to 60 m in length. Humans have used seaweeds for about 14,000 years and as food products by the Japanese and Chinese cultures for centuries; the Aztec population also collected and cultivated seaweeds (McHugh, 2003; Pulz and Gross, 2004; Dillehay *et al.*, 2008). Their applications in the food industry have been increasing, as well as in the textile, pharmaceutical, cosmetic, and biotechnology industries (Bartsh et al., 2008). Seaweed biomass is used for the production of phycocolloids such as agar-agar, alginates, and carrageenan. More than 200 species are utilised worldwide, but the most cultivated and used are the brown algae *Laminaria japonica* and *Undaria pinnatifida*; the red algae *Porphyra sp.*, *Eucheuma*, *Kappaphycus*, and *Gracilaria*; and the green algae *Monostroma* and *Enteromorpha* (Lüning & Pang, 2003; Machu *et al.*, 2015; Hoang *et al.*, 2015; Peteiro *et al.*, 2016; Bito *et al.*, 2017; Chan & Mantanju, 2017; Pramanick *et al.*, 2017; Ghani *et al.*, 2018; Huang & Yang, 2019). Edible algae mainly contain protein, carbohydrates and minerals (Rupérez, 2002; Kumar *et al.*, 2015; Jung *et al.*, 2016; Admassu *et al.*, 2018; Wang *et al.*, 2018). Among them *U. pinnatifida* and *Porphyra purpurea* have high protein content. Studies have shown that the protein content in edible algae could be as high as 45.7% (Fujiwara-Arasaki *et al.*, 1984). Edible algae can also provide some essential fatty acids, such as linoleic acid and linolenic acid (Smit, 2004) and ecosapentaenoic acid (EPA) (Blouin *et al.*, 2006; Campos *et al.*, 2019). Goñi and colleagues. (2002) suggested that edible algae polysaccharides are a good source of dietary fiber. They also contain various minerals and vitamins which are important for the health (Watanabe et al., 2002; Smit, 2004; Cofrades et al., 2013). Elemental composition, which is composed by structural elements, macroelements, trace elements and ultra trace elements. Previous studies reported benefits to the consumption of seafood related with its content of essential minerals. Structural elements are the basis of human body: carbon, oxygen, hydrogen, nitrogen, sulphur and phosphorous. Regardless, essential minerals are the definition for all elements that cannot be produced by the organism; they must become from human diet and are crucial for life maintenance (Oehlenschlăger, 1997). Macroelements are considered essential: Na, K, Ca and Mg. They have as main functions the maintenance of the colloidal system, regulation of the acid-base balance and intervene in the skeleton structure (Lourenço et al., 2012). Essential elements Ca, K, Mg, Na and the structural element P have important functions in: balance between intracellular ions (Lall, 1995), structure, acid-base reactions and maintenance (Lourenço, 2011). Other elements can be found in marine products in lower levels (mg or µg per kilogram). They are called the trace elements: Cu, Fe and Zn and ultra trace elements, for example: Mn and Se (Lall, 1995). Trace and ultra trace elements can promote toxicity if present in high levels (Belitz *et al.,* 2004).

Iron, zinc, copper and manganese, selenium have important functions to maintain health. One of their functions is influencing enzymes with catalytic functions (Raimundo *et al*., 2004).

The enclosure of macroalgae or their extracts to food products helps in reducing the utilization of chemical preservatives (Gupta et al., 2011). Edible seaweeds contain diverse bioactive compounds with potential health benefits and their use as functional ingredients opens new prospects for food processing (Kılınç *et al*., 2013). Some macroalgae polysaccharides are used by food industry as texture modifiers because of their high viscosity and gelling properties (Kılınç *et al*., 2013). In Asia, seaweeds have been used for centuries in salads, soups and as low calorie dietetic foods. The dietary fiber which constitutes 25-75 % of the dry weight (DW) of marine algae and represents their major component as primarily soluble fiber (Jiménez-Escrig & Sánchez-Muniz, 2000).

In the food industry, seaweeds have been introduced to meat and bakery products (Gupta et al., 2011) that contain an insufficient amount of dietary fiber and an excessive amount of sodium that maybe harmful for humans. On the other hand, the addition of seaweeds as a functional component may help overcome technological problems in meat products associated with a low salt and fat contents and water binding properties. Some attempts were made to develop model systems of meat emulsions with low fat and salt content, with an addition of various bioactive compounds from marine seaweeds. Meat enrichment with seaweeds supplied soluble polyphenolic compounds, which increased the antioxidative potential of the whole system (López-López *et al.* 2009). The addition of *U. pinnatifida* on beef patties reduced thawing and cooking losses as well as improved the product's texture (López-López *et al.* 2010). Sasaki and colleagues (2008) found that fucoxanthin, the main carotenoid pigment in *U. pinnatifida*, improved the appearance and extended the shelf life (reducing the peroxidation of lipids) of chicken meat products. The algae *Fucus vesiculosus* was added to fish and fish products (Wang et al. 2010) and Senthil and colleagues (2005) used *Eucheuma* powder as an ingredient of a fish cutlet. It was found that the addition of up to 10% algae had no negative effect on appearance, texture and acceptability. Athukorala and co-authors (2003) reported that the inclusion of marine red algae *(Grateloupia filicina*) extract (0.05%) in fish oil inhibited lipid oxidation, as well as Oucif and associates (2018), that found the effectiveness of a combined ethanol–aqueous extract of alga *Cystoseira compressa* for the quality enhancement of a chilled fatty fish species.

Regarding cereal-based products, study with the edible seaweed *Undaria pinnatifida*, rich in fucoxanthin was conducted and concluded that pasta with 10% of algae was sensory acceptable (a mild taste of algae was perceived, but it was still close to the control sample). Moreover, the presence of seaweeds supported the interaction between starch granules and the protein matrix, which resulted in higher quality pasta (Prabhasankar et al., 2009a). Pasta containing Indian brown seaweed *Sargassum marginatum* was also developed. Supplementation with algae improved its bio-functionality and quality (Prabhasankar et al., 2009b). Also Chinese fresh egg noodles were created with an addition of green algae *Monostroma nitidum* (Chang & Wu, 2008). Bread is another cereal-based product which quality could be improved through the addition of green seaweeds *Ulva lactuca*, powdered *Laminaria* seaweed (Cofrades *et al.*, 2013) and *Ascophyllum nodosum* (Hall et al. 2012), creating a functional bread. The consumption of this bread at breakfast led to a significant decrease (16.4%) of energy intake 4 hours later due to the fiber content that increase satiety (Hall et al. 2012). Supplementation with seaweeds like *Porphyra tenera* decreased the rate of starch hydrolysis, and also decreased the glycemic response to white bread in healthy volunteers (Goñi *et al.*, 2000) Seaweeds-based flour and lipid powders are currently widely discussed, as they are an element of modern cuisine, also appreciated in the vegan market, since they are used instead of eggs (Pina-Pérez *et al.*, 2017).

Given that seaweeds are plentiful but a poorly exploited marine food resource (FAO, 2016), it is probable that more emphasis will be placed on the use of seaweeds for human consumption in the future to face the challenge of seeking new food and sustainable resources with a lower carbon footprint. So, it is necessary to raise awareness about benefits of edible seaweeds consumption, in order to promote its use in Western food habits, in particular in countries where consumption is still low, as is the case of Portugal. However, the presence of deleterious substances in food is often inevitable as they may occur naturally. Therefore, the potential hazards of edible seaweeds should be evaluated. Special attention must be given to arsenic (As) since several studies evidence that macroalgae have high capacity to accumulate this element (CEVA, 2014; EC, 2018).

Thus, this work intends to characterise the nutritional profile of some of the most consumed seaweeds species that occur in the Portuguese coast such as *Undaria pinnatifida*, *Porphyra linearis*, and *Porphyra umbilicalis*, in order to evaluate their potential for human consumption.

2. Methodology

2.1 Sample collection

Seaweeds *U. pinnatifida, P.linearis* and *P.*umbilicalis were collected in the beach of Buarcos (Figueira da Foz) during June of 2018. The seaweeds were cleaned in the beach with seawater and at laboratory (Coimbra) were identified, washed again with tap water, drained and stored in plastic bags at – 20 °C. The transportation to the laboratory at Lisbon was done in refrigerated environment, in order to avoid defrosting. Seaweeds were freeze-dried for at least 24 hours and were weighed in a precise and reliable balance with accurate of 0.001g (Mettler Toledo GMbH) before and after freeze drying. Dried seaweeds were grounded to a homogenous powder and stored at -80 °C until analyses be performed.

2.2 Analytical methods

All the analyses were performed on freeze-dried samples in duplicate, except for fatty acids composition and for the elements determined by Energy Dispersive X-ray Fluorescence (EDXRF) that were done in triplicate. The ultrapure water used was produced on equipment from Millipore (Q Plus Millipore). The results are given on a dry basis (dry weight - DW), except in the case of nutritional contribution (NC) for which wet basis (wet weight - WW) was used.

2.2.1 Proximate composition

The content of moisture, ash and protein were performed according to reference methods described in AOAC (1998). Moisture was obtained by gravimetric method. Samples were weighed and dried at 105 ± 2°C in an oven for 12 hours, dried in a desiccator and weighed until constant weight. The moisture of fresh samples was estimated by weighing the samples before and after being freeze-dried.

The ash content was determined by gravimetric method. The samples were dried at 105 ± 2°C and then dry-ashed in a furnace (HERAEUS Electronic) at 500 ± 25 °C overnight, dried in desiccators and weighed until constant weight.

The amount of protein was determined as total nitrogen x 6.25. Total nitrogen was quantified on Leco FP 528 equipment. The nitrogen was released, on the presence of oxygen, during the combustion at 850 °C. The conversion value of 6.25 (FAO, 2003) was used to obtain the protein content.

The extraction of total lipids was done according to Folch and co-authors (1957) only on samples of *U. pinnatifida* and *P. linearis*. Chloroform/methanol ($CHCl_3$:MeOH) mixture was added and the samples were placed in a water bath and then hydrochloric acid (HCl) and magnesium chloride ($MgCl_2$) were added (to precipitate proteins and migration to the organic phase). The mixture was centrifuged at 2000 *g* for 10 min at 4 °C to obtain two different phases: organic and aqueous. Organic phase was removed to another tube and $CHCl_3$:MeOH was added to the residue. The centrifugation and collection of organic phase was repeated so it can be mixed with the first collection. The organic solvent was evaporated through a nitrogen atmosphere and after this process the tubes were weighed. The total lipids (fat) content were calculated by weight difference.

Energy value was calculated using the following formula, expressed in Kcal / 100 g (FAO, 2003):

Energy value (Kcal / 100 g) = (F% × 9) + (P% × 4) + (CH% × 4)

F%: fat percentage;

P%: percentage of protein.

CH%: Carbohydrates

2.2.2 Fatty acids profile

The fatty acids profile was determined by the analysis of methyl esters, prepared according to the method described by Bandarra and colleagues, (1997). Transmethylation of the fatty acids by acid catalysis was performed by adding the acetyl chloride:methanol solution. The esterification was carried out for one hour, in a water bath at 80 °C, and after cooling, ultrapure water and n-heptane were added. The mixture was centrifuged at 2000 *g* for 5 min and the organic phase was collected into a vial by filtration through a cotton column containing anhydrous sodium sulfate. The separation and detection of the fatty acid methyl esters was accomplished in a gas chromatographer (Varian Star CP 3800 GC, Walnut Creek, CA), equipped with an auto-sampler and a flame ionization detector (FID). Separation of the methyl esters was carried out on a capillary polyethylene glycol column (DB-WAX, Folsom, CA) (30 m × 0.25 mm internal diameter, film thickness: 0.25 µm) using helium as the carrier gas, maintained at 180 °C for 5 min, followed by a heating ramp of 4 °C/min for about 10 min, until the temperature reached 220 °C which was maintained for 25 min. Separate compounds were detected on the FID detector at 250 °C. Fatty acids identification and quantification were performed using calibration curves with the standards Sigma-Aldrich/Supelco PUFA No.1, Marine Source - Ref. 47033 and PUFA No.3, Menhaden oil - Ref. 47085-U. The chromatograms obtained give the relative percentage of each fatty acid present in the sample.

2.2.3 Elemental composition

The method for the determination of Cu, Fe, K, Mg, Mn, Na and Zn content was performed in accordance with Jorhem (2000) by flame atomic absorption spectrometry

(Spectr AA 55B, Varian). First, ash was obtained from the sample that was dissolved in heated nitric acid in the crucible. The solution obtained was filtered and read at the absorption wavelength of each element. The detection limit of this method is 0.01 (K and Mn); 0.02 (Cu and Mg); 0.06 (Zn); 0.32 (Fe) and 0.09 (Na) mg/kg.

Phosphorus content was determined according to ISO 13730 (ISO, 1996) by spectrophotometery. All material used was free of detergents with phosphates. Samples were incinerated (500 ± 25 °C) and hydrolysed with nitric acid followed by filtration and dilution. The solution was mixed in a balloon with colorimetric reagent (aqueous solution composed by ammonium monovanadate, ultrapure nitric acid and ammonia heptamolybdate). The absorbance was measured at a wavelength of 430 nm (UV/Vis UV2 spectrophotometer, UNICAM). The content of P was calculated from the content of phosphorous pentoxide (P_2O_5). The limit of detection is 0.01 mg/kg.

The quantification of Ca, Se and total As was carried out by EDXRF method using a portable XRF analyser (Niton model XL3t 950 He GOLDD+, Thermo Scientific USA), under Helium atmosphere. Each sample was analysed, applying the maximum measurement time (360 seconds). The limit of detection is 259, 2.6 and 3.8 mg/kg for Ca, Se and As, respectively.

Determination of Cd and Pb content were done according to NP EN 14084 (CEN, 2003) by graphite furnace atomic absorption spectrometry (Spectr AA 220Z, Varian). The digestion of samples was done with nitric acid (65%) and hydrogen peroxide (30%) in a specific microwave (CEM, MARS 5) at 210 °C (temperature rising slowly) during 15 minutes. Then, the digested solution was transferred to a volumetric balloon and diluted with ultrapure water. The detection limit is 0.002 and 0.02 mg/kg, while the limit of quantification is 0.006 and 0.06 mg/kg for Cd and Pb, respectively.

The determination of total Hg content was based in EPA 7473 (EPA, 2007) and it was done through atomic absorption spectrophometric method using a direct mercury analyser (AMA 254, Leco). Samples were thermally decomposed in the furnace (at approximately 750 °C). Amalgamation to release mercury vapor was conducted. The absorbance was measured by atomic absorption spectrometry. The detection limit of the method was 0.004 mg/kg and the limit of quantification was 0.011 mg/kg.

2.3 Statistical analysis

All data of this study was compared using the software STATISTICA 6.1 (Stat soft, Inc., Tulsa, OK7410, USA). One way ANOVA followed by Tukey test was used to assess differences on the results among the three species of seaweeds. The Student's *t*-test was applied to compare total lipids and carbohydrates contents between *P. linearis* and *U. pinnatifida*. Differences with p-value lower than 0.05 were considered statistically significant.

3. Results and Discussion

The knowledge of nutritional profile and toxic elements of *U. pinnatifida, P. linearis* and *P. umbilicalis* is crucial to assure their benefits and risks for human consumption. The results obtained for those seaweeds are shown in Tables 1-5. They were reported on dry weight (DW) in mg/kg, except the proximate composition which is expressed in g/100 g.

Fresh seaweeds are composed by 80-90% of moisture (da Costa, 2014). Otherwise, moisture content (Table 1) determined in dry weight, in this study ranged between 4 - 13 %. Similar results were obtained by da Costa (2014) for macroalgae, including *U. pinnatifida* and *Porphyra tenera*. The three seaweeds studied had different moisture content due to the incomplete freeze-drying. The average moisture level of fresh seaweeds was around 82.5%. These values are similar to those obtained for *Porphyra* seaweeds by Paiva and co-workers (2014).

The ash content gives an estimate of the mineral content of the matrix in study (da Costa, 2014). The ash content of our seaweeds (Table 1) ranged from around 15 (reported for *Porphyra* species) to 23 % (*U. pinnatifida*). This variation is in accordance with the range reported in other studies and is related with the geographic origin and species (Rupérez, 2002; Sánchez-Machado *et al*., 2004). Furthermore, brown seaweed *U. pinnatifida* presented higher ash content than the two red seaweeds, which is in agreement with Rupérez (2002), Sánchez-Machado *et al*. (2004), and Taboada *et al*. (2013).

Table 1. Proximate composition (g/100g DW) and caloric value (Kcal/100g) of *U. pinnatifida*, *P. linearis* and *P. umbilicalis*.

	g/100g					Kcal/100g
Seaweed	Moisture	Ash	Protein	Lipids	Carbohydrates[1]	Caloric value
U. pinnatifida	6.43±0.06[a]	23.06±0.15[a]	25.43±0.33[a]	2.93±0.07[a]	42.15±0.36[a]	296.69
P. linearis	3.52±0.41[b]	14.84±0.45[b]	33.30±0.70[b]	1.82±0.10[b]	46.52±0.26[b]	335.66
P. umbilicalis	13.41±0.07[c]	15.36±0.38[b]	27.36±0.41[a]	ND[2]	ND[3]	ND[4]

The values are mean ±standard deviation

In each column, different superscript letters correspond to significant differences ($p<0.05$)

[1]Obtained by difference (FAO, 2003)

[2]not determined due to insufficient sample amount

[3]not estimated due to the lack of information regarding lipids content

[4]not estimated due to the lack of information regarding lipids and carbohydrates contents

Proteins have various functions: structural, sarcoplasmic (enzymes) and connective (present in tissues) (Huss, 1995; Belitz *et al*., 2004; Wang and Tang, 2017). The protein content of analysed seaweeds (Table 1) ranged from 25 to 33 %, in concordance with Sánchez-Machado *et al*. (2004). Furthermore, these results are similar to protein content of Azores seaweeds (*Porphyra* spp.), which was 25% (Paiva *et al*., 2014) and 26% (Patarra *et al*., 2010). Of all the seaweeds studied, the highest protein level was verified in *P. linearis*, in accordance with earlier investigations (McHugh, 2003).

The total lipids content (Table 1) was about 1.8 - 2.9 %, which is a small amount as expected. The values obtained in our study are in agreement with Cherry et al. (2019) (0.3 – 8.9 %). Generally, the amount of total lipids in algae is limited (Dawczynski *et al*., 2007), which is similar to other seafood for like cod, horse mackerel, shrimp and octopus (Nunes et al., 2008). It is usual that brown seaweeds have higher lipids content than red seaweeds (Gosh et al., 2015).

The amount of total carbohydrates can vary from 35 to 76% (Holdt & Kraan, 2011), which is in line with our results of 42.15% for *U. pinnatifida* and 46.52% for *P. linearis*. Among the carbohydrates, non-starch polysaccharides such as carrageenan, alginate and agar are the most abundant food elements of seaweeds, being the antioxidant activity associated with agar (Holdt & Kraan, 2011).

Generally, the macroelements profile (Tables 2 and 3) in this study was K>Na>Ca>Mg>P (Table 2). The Mg, Na, K and P content was higher in the brown seaweed *U. pinnatifida* than in red ones (*P. linearis* and *P.umbilicalis*), with significant differences. Otherwise, the macroelement Ca is the only that revealed statistical differences among the three species and is higher in *P. umbilicalis.*

The average K content of the three studied seaweeds ranged between 25152 and 48781 mg/kg, which are higher when compared with other literature. For example, in the study conducted by Paiva and co-workers (2014) the content of 24811mg/Kg was found for K in *Pophyra* spp.

Table 2. Macroelements (mg/kg DW) of *U. pinnatifida*, *P. linearis* and *P. umbilicalis*.

	Macroelements				
Seaweed	Ca**	K	Mg	Na	P
U. pinnatifida	10386±180[a]	48781±3400[a]	9721±111[a]	29324±3228[a]	6207±121[a]
P. linearis	1608±125[b]	32242±415[b]	3866±18[b]	11104±193[b]	5527±138[b]
P. umbilicalis	13862±117[c]	25152±264[b]	3578±73[b]	10982±2599[b]	4769±77[c]

The values are mean ±standard deviation.

**EDXRF method

In each column, different superscript letters correspond to significant differences ($p<0.05$).

Among all the trace elements (Table 3), Fe content had the highest results and Cu the lowest. Globally, the red seaweeds had higher trace elements content than brown seaweed, in opposition to what was verified for the macroelements. Same species of studied red seaweeds presented similar trace elements content, except of Zn values.

The analysis of data (Table 3) revealed that the average of Fe content ranged from 102 to 134 mg/kg for *U. pinnatifida* and *P. linearis,* respectively. In agreement to the results reported by da Costa (2014) and Rupérez (2002)**,** the red seaweeds analysed in the present study had similar Fe content compared with the brown seaweed. However, these values were all lower than the Fe content observed in *Gracilaria vermiculophylla* from other study (Cabrita *et al*, 2016) and higher than *Gracilaria gracilis* (Rodrigues *et al*., 2015), both red seaweeds. Differences between species may be observed in different studies on seaweeds depending on geographic location, environment and seasonal variations (Mišurcová *et al*., 2011).

Regarding the Zn level, this was distinct among all the samples, with higher values in *P. linearis*. The brown seaweed *U. pinnatifida* presented the lowest Cu value when compared with the two red seaweeds.

Regarding the Mn content, the differences observed between brown and red seaweeds were reported before in Rubio *et al*., (2017).

Table 3. Trace and ultra trace elements (mg/kg DW) of *U. pinnatifida*, *P. linearis* and *P. umbilicalis*.

	Trace Elements			Ultra trace Elements	
Seaweed	Cu	Fe	Zn	Mn	Se
U. pinnatifida	1.02±0.33[a]	102.16±3.06[a]	17.58±0.61[a]	8.28±0.44[a]	<3,6*
P. linearis	2.71±0.16[b]	133.76±4.63[b]	61.08±0.07[b]	34.81±0.02[b]	<3.6*
P. umbilicalis	2.46±0.15[b]	127.21±7.74[b]	54.89±0.57[c]	33.00±0.75[b]	<3.6*

The values are mean ± standard deviation

*LD = detection limit. EDXRF method

In each column, different superscript letters correspond to significant differences ($p<0.05$).

The concentration of As, Cd, Hg and Pb with toxicological potential is shown in Table 4. Seaweeds have a large capacity to accumulate various heavy metals, and they have been used as bioindicators of the marine environmental contamination (Riget *et al.*, 1997). The contamination by these elements is connected to the safety of the consumer regarding the ingestion of this type of food (Almela *et al.*, 2006). Australia, New Zealand and France have established a set of legal standards regarding the presence of toxic elements in edible seaweeds (ANZFSC, 2011; CEVA, 2014), respectively.

In respect to our results, the brown seaweeds, frequently, have high content of elements that can be toxic (Mouritsen *et al.*, 2013; Cherry et al., 2019). Cadmium, Pb and Hg have no essential function whereas if ingested during long periods in low concentration, they are toxic. Some consumers do not pay attention to their presence and do not see them as a risk (Oehlenschlǎger, 2002). Cd, Hg and Pb have no-essential functions and may reflect the pollution in oceans (Belitz et al., 2004; Carvalho 2005). Cadmium may compete with other elements: Cu, Fe and Zn, compromising their absorption, which can be a serious problem (Carvalho *et al.*, 2005). It influences the normal function of kidneys

(Wilk *et al*., 2017), so their amount must be controlled through legislation. In this study Cd content (Table 4) had significant differences among the three seaweeds. The results of *U. pinnatifida* and *P. umbilicalis* were below the European established limit for mollusks, 1mg/kg WW (EC, 2006). These values of *U. pinnatifida* and *P. umbilicalis* were also below the French limit of 0.5 mg/kg DW (CEVA, 2014) and consistent with the results obtained in literature (Almela et al., 2006). *P. linearis* exceeded the Cd limit establish by French legislation.

Other heavy metal that is known to be present in seafood and human food chain is mercury being this fact of great concern due to its high neurotoxicity (Plessi et al., 2001). The excessive consumption leads to a negative effect in the central nervous system (Assi et al., 2016). However the Hg content was only found for *U. pinnatifida*. This level is in line with the established limits of 0.1 mg/kg DW (CEVA, 2014). These Hg values do not represent a risk for human's ingestion. Regarding the Pb level it was only quantified in *P. umbilicalis*. The result agreed with French legislation, being the limit of 5 mg/kg DW (CEVA, 2014).

Table 4. Heavy metals composition (mg/kg DW) of *U. pinnatifida*, *P. linearis* and *P. umbilicalis.*

Seaweed	As*	Cd	Pb	Hg
U. pinnatifida	46.79±0.35[a]	0.32±0.01[a]	<0.06**	0.02±0.00
P. linearis	54.87±0.42[b]	1.24±0.05[b]	<0.06**	<0.011**
P. umbilicalis	46.69±0.72[a]	0.13±0.01[c]	0.10±0.02	<0.011*

The values are mean ± standard deviation.

*EDXRF method

**LQ: limit of quantification.

In each column, different superscript letters correspond to significant differences ($p<0.05$).

Regarding to the presence of As in human chain, this factor occurs due to pollution, insecticides and herbicides (Hughes *et al*., 2011). When ingested, if it is not metabolised, it has no toxicity (Shaw & Liu, 2000) and is eliminated unchanged (Francesconi, 2010). Arsenic can be toxic, depending on its form (organic or inorganic). The inorganic form is the most dangerous (Hughes *et al*., 2011), which is associated with the damage of healthy cells that leads to cancer (Cherry et al., 2019). In concerning to this toxicity, the EFSA identifies inorganic As values for fish and other marine foods of 0.03 and 0.1 mg/kg, respectively, as realistic levels for calculating human exposure through diet, although it also mentions that the high consumption of algae-based products may increase exposure to inorganic As to values of 4 µg/kg body weight per day (EFSA, 2009). In our work only the total arsenic was quantified and there were significant differences between *P. linearis* and the other two seaweeds. The three seaweeds had similar As results with the values reported by Taylor et al. (2017) for *U. pinnatifida* (34.7 mg/kg). Considering that total As contents are higher than the limit establish for inorganic As in France (3mg/Kg DW) (CEVA, 2014). The determination of the toxic form of arsenic is crucial in further studies.

Other element that can be dangerous associated with seaweed consumption is the iodine when is present in high concentrations. Due to insufficient sample it could not be determined although in further studies it should be analysed (Holdt and Kraan, 2011).

In our study, the total fatty acid methyl esters (FAME) (Table 5) was analysed for the three seaweeds as well as the total of different types of fatty acids (saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and n3 PUFA/n6 PUFA ratio. Their composition are usually low in seaweeds (Fleurence *et al*., 1994; Sánchez-Machado *et al*., 2004; Dawczynski *et al*., 2007), this value can change with season, localization (Rodrigues *et al*., 2015) or can vary among different species of seaweeds (Schmid *et al*., 2018). As we can observe in the present study, brown seaweeds generally have more total fatty acid concentration (SFA, MUFA and n-6) than red ones, in accordance with Sánchez-Machado *et al.* (2004) and Schmid *et al.* (2018). In reference to total SFA, the results oscillated between 35 % (*P. umbilicalis*) and 40 % (*U. pinnatifida*), which is in line with other studies on seaweeds of *Porphyra* genus (Schmid *et al*., 2014). The highest result was obtained for palmitic acid. Red seaweeds only had the stearic acid higher than the brown seaweed. Similar values of this fatty acid content were found in Blouin *et al.* (2006) for *P. umbilicalis.*

Seaweeds can be interesting because of their content of omega-3 (Sioen *et al*., 2007) and omega-6 polyunsaturated fatty acids, which decrease the probability of illness and consequently decreasing the morbility and mortality (Calder & Grimble, 2002). Regarding the average of PUFA content, the most abundant of the three seaweeds were the n-6, arachidonic. In reference of omega-3, docosahexaenoic acid (DHA) was not found in seaweeds, which is in agreement with the results of other researches (Fleurence *et al*., 1994; Cherry *et al*., 2019). In addition the EPA level is associated with the increasing of PUFA n3/PUFA n-6 ratio. This association seems to be supported by other literature (Mouritsen *et al*., 2013; Schmid *et al*., 2014).

Table 5. Percentage of fatty acids composition (% of total fatty acids) of *U. pinnatifida*, *P. linearis* and *P. umbilicalis*.

FAME	*U. pinnatifida*	*P. linearis*	*P. umbilicalis*
Myristic (14:0)	5.15 ± 0.35[a]	0.82 ± 0.12[b]	0.17 ± 0.01[b]
Palmitic (16:0)	23.95 ± 0.31[a]	27.38 ± 0.53[b]	25.79 ± 0.34[c]
Stearic (18:0)	1.45 ± 0.05[a]	0.55 ± 0.01[b]	0.59 ± 0.00[b]
Total SFA	39.66 ± 0.58[a]	36.90 ± 0.67[b]	34.65 ± 0.10[b]
Palmitoleic (16:1, n-7)	0.82 ± 0.05	ND	ND
Oleic (18:1, n-9)	10.83 ± 0.25[a]	2.16 ± 0.06[b]	2.18 ± 0.01[b]
Vaccenic (18:1, n-7)	ND	0.40 ± 0.01[a]	0.38 ± 0.01[b]
Total amount of MUFA	11.93 ± 0.25[a]	5.73 ± 0.10[b]	5.66 ± 0.12[b]
Linoleic (18:2, n-6)	5.19 ± 0.09[a]	1.18 ± 0.04[b]	1.24 ± 0.02[b]
Alpha-linolenic (18:3, n-3)	5.85 0.20	ND	ND
Stearidonic (18:4, n-3)	9.79 0.07	ND	ND
Eicosatetraenoic (20:4, n-3)	0.50 ± 0.02[a]	0.81 ± 0.01[b]	0.88 ± 0.02[c]
Arachidonic (20:4 n-6)	10.71 ± 0.32[a]	11.18 ± 0.24[a]	11.19 ± 0.19[a]
Eicosapentaenoic (20:5, n-3)	5.52 ± 0.26[a]	39.09 ± 0.64[b]	40.24 ± 0.32[c]
Docosahexaenoic (22:6, n-3)	ND	ND	ND
Total PUFA	38.22 ± 0.84[a]	53.54 ± 0.88[b]	54.80 ± 0.47[b]
Total amount of n-3	22.32 ± 0.47[a]	40.45 ± 0.65[b]	41.71 ± 0.31[c]
Total amount of n-6	15.90 ± 0.39[a]	12.36 ± 0.26[b]	12.42 ± 0.18[b]
Ratio n-3/n-6	1.40 ± 0.02[a]	3.27 ± 0.05[b]	3.36 ± 0.03[b]

The values are mean ± standard deviation.

*ND = not detected.

In each row, different superscript letters correspond to significant differences ($p<0.05$).

The *U. pinnatifida* revealed a lower n3 PUFA/n6 PUFA ratio than the red seaweeds, with statistical significant differences, which is in line with the literature (Paiva *et al*., 2014; Rodrigues *et al*., 2015; Campos *et al*., 2019). This n3/n6 ratio is crucial to increase the immunity system and decrease the probability of inflammation and to maintenance the elements of cell membrane (Calder & Grimble, 2002).

Based on references values, the potential contribution of analysed seaweeds to daily dietary demands is indicated in Tables 6 to 8.

To highlight the nutritional contribution (NC) of seaweeds and their consumption risks, the values of macronutrients, macroelements and contaminants where converted in 25 grams of fresh seaweed (WW). This conversion was done through the utilization of conversion factors (100 - % moisture WW / 100 - % moisture DW): 0.1870, 0.1813 and 0.2021 for *U. pinnatifida*, *P. linearis* and *P. umbilicalis,* respectively. Seaweeds quantity was reduced to 25 grams because it was the amount of seaweed (*U. pinnatifida*) used to make a salad in Spain (González et al., 2019), so it can give us a real perspective. The nutritional contribution (%) was calculated through the formula: elements content in 25g x 100 / DRV (dietary reference value).

The energy values ranged between 0.54 and 0.73 % of the recommendation. On the other hand, it reveals a low lipid content, which gives an advantage since it can be part of a balanced diet.

Table 6. Composition on macronutrients in a 25 g portion of fresh seaweeds (g/25g WW), calories (Kcal/25g), dietary reference values (DRV) and nutritional contribution (%) of *U. pinnatifida*, *P. linearis* and *P. umbilicalis*.

Macronutrients		DRV	*U. pinnatifida*	NC (%)	*P. linearis*	NC (%)	*P.umbilicalis*	NC (%)
Proteins		53[1]	1.19	2.25	1.51	2.85	1.38	2.60
Lipids	Men	20 - 35[2]	0.14	0.05	0.08	0.03	-	-
	Women	20 - 35[2]	0.14	0.06	0.08	0.03		
Carbohydrates[3]	Men	45 - 60[2]	1.97	0.53	2.11	0.57	-	-
	Women	45 - 60[2]	1.97	0.66	2.11	0.71		
Energy[4]	Men	2579	13.88	0.54	15.21	0.59	-	-
	Women	2078	13.88	0.67	15.21	0.73	-	-

The results were calculated from the mean values.

[1]PRI: Population reference intake (g/day) for adults (≥ 18 years old with reference weight = 63.3 kg) (EFSA, 2017)

[2]Percentage of total energy intake, (EFSA, 2017)

[3]Obtained by difference (FAO, 2003)

[4]Average Kcal/day for moderately active individuals, 18-59 years old (EFSA, 2017)

Regarding the contribution of fresh seaweeds in macroelements, it is known that seaweeds can be rich in minerals, however it depends on their source and season. In this study small nutritional contribution was observed for one meal (25g) like in China, Japan and South Korea (Cherry et al., 2019). Only red seaweeds had a result over 15% in Zn and Mn (Table 7). Otherwise, the Na result shows that the studied seaweeds can be a good option for individuals who suffer from hypertension (Paiva et al., 2014).

Table 7. Composition on macroelements in a 25 g portion of fresh seaweeds (mg/25g WW), dietary reference values (DRV) and nutritional contribution (NC) in *U. pinnatifida*, *P. linearis* and *P. umbilicalis*.

Macroelements	Gender	DRV	*U. pinnatifida*		*P. linearis*		*P.umbilicalis*	
			mg/25g	NC (%)	mg/25g	NC (%)	mg/25g	NC (%)
Ca	Men/Women	950[1]	48.55	5.11	7.29	0.77	70.04	7.37
K	Men/Women	3500[2]	228.05	6.52	146.14	4.18	127.08	3.63
Mg	Men	350[2]	45.45	12.99	17.52	5.00	18.07	5.17
	Women	300[3]	45.45	15.15	17.52	5.84	18.08	6.03
Na	Men/Women	2000[3]	137.09	6.85	50.33	2.52	55.49	2.77
P	Men/Women	550[2]	29.02	5.28	25.05	4.55	24.10	4.38

The results were calculated from the mean values

[1]PRI: population reference intake (mg/day) for adults (≥ 25 years old) (EFSA, 2017)

[2]AI: adequate intake (mg/day) for adults (≥ 18 years old) (EFSA, 2017)

[3] AI: adequate intake (mg/day) for adults (≥ 18 years old) (EFSA, 2019)

According to recommended values of trace and ultra trace elements (Table 8) the *U. pinnatifida* had the lowest results. Red seaweeds had the highest results, especially for Zn and Mn.

Table 8. Composition on trace and ultra trace elements in a 25 g portion of fresh seaweeds (mg/25g WW), dietary reference values (DRV) and nutritional contribution (NC) in *U. pinnatifida*, *P. linearis* and *P. umbilicalis*.

Trace and ultra trace Elements		Gender	DRV	*U. pinnatifida*		*P. linearis*		*P. umbilicalis*	
				mg/25g	NC (%)	mg/25g	NC (%)	mg/25g	NC (%)
Trace Elements	Cu	Men	1.60[1]	0.005	0.30	0.012	0.77	0.012	0.78
		Women	1.30[1]	0.005	0.37	0.012	0.94	0.012	0.96
	Fe	Men*	11.00[2]	0.478	4.34	0.606	5.51	0.643	5.84
		Women**	16.00[2]	0.478	2.99	0.606	3.79	0.643	4.02
	Zn	Men***	12.85[2]	0.082	0.64	0.277	2.16	0.277	2.16
		Women***	10.13[2]	0.082	0.81	0.277	2.73	0.277	2.73
Ultra trace Elements	Mn	Men / Women	3.00[1]	0.039	1.29	0.158	5.26	0.167	5.56

The results were calculated from the mean values

[1]AI: adequate intake (mg/day) for adults (≥ 18 years old) (EFSA, 2017)

[2]PRI: population reference intake (mg/day) for adults (≥ 18 years old) (EFSA, 2017)

*Men and women postmenopausal

** Women premenopausal

*** DRV corresponds to the average value according with phytate intake

4. Conclusions

The analysed seaweeds are a sustainable and very complete food due to the presence of various important compounds. The K and Fe were respectively the macro and micro elements, with the highest content.

The most relevant NC´s were found for Ca (7.37% in *P. umbilicalis*), Mg (12.99% for men and 15.15% for women in *U. pinnatifida*), Fe (5.84% for men and 4.02% for women in *P. umbilicalis*) and Mn (5.56% in *P. umbilicalis*).

These seaweeds can be a part of a balanced diet due to their low lipids content and low calories. Additionally, they present high PUFA and EPA values and a good n3 PUFA/n6 PUFA ratio.

Regarding the elements that can be toxic, the results show the need to analyse inorganic As and to establish limits in Europe. In relation to Cd, Pb and Hg the contents are lower than the limits. Iodine is other element that is important to establish its quantity in seaweeds due to its potential toxicity.

Further studies on seaweeds contaminants are needed, once they have an elevated absorption capacity. It is also interesting to study these seaweeds in different seasons to understand their influence in nutritional and toxicological composition.

References

Admassu, H., Abera, T., Abraha, B., Yang, R., & Zhao, W. (2018). Proximate, Mineral and Amino acid Composition of Dried Laver (Porphyra spp.) Seaweed. *Journal of Academia and Industrial Research (JAIR), 6*(9), 149.

Almela, C., Clemente, J., Vélez, D e M. Rosa. (2006). Total arsenic, lead and cadmium content in edible seaweed sold in Spain. *Food and Chemistry Toxicology*, 44: 1901-1908.

ANZFSC (Australia New Zealand Food Standards Code). (2011). Australia New Zealand Food Standards Code: Standard 1.4.1: Contaminants and Natural Toxicants. http://www.comlaw.gov.au/Details/F2011C00121.

Assi, M. A., Hezmee, M. N. M., Haron, A. W., Sabri, M. Y. M., & Rajion, M. A. (2016). The detrimental effects of lead on human and animal health. *Veterinary World*, *9*(6), 660.

AOAC (Association of Oficial Analytical Chemists International). (1998). *Official Methods of Analysis*. (16th ed.). Washington DC: AOAC.

Athukorala, Y., Lee, K. W., Shahidi, F., Heu, M. S., Kim, H. T., Lee, J. S., & Jeon, Y. J. (2003). Antioxidant efficacy of extracts of an edible red alga (Grateloupia filicina) in linoleic acid and fish oil. *Journal of Food Lipids*, *10*(4), 313-327.

Bandarra, N. M., Batista, I., Nunes, M. L., Empis, J. M., & Christie, W. W. (1997). Seasonal changes in lipid composition of sardine (Sardina pilchardus). *Journal of Food Science*, *62*(1), 40-42.

Bartsch, I., Wiencke, C., Bischof, K., Buchholz, C. M., Buck, B. H., Eggert, A., ...& Karsten, U. (2008). The genus *Laminaria sensu lato*: recent insights and developments. *European Journal of Phycology*, *43*(1), 1-86.

Belitz, H. D., Grosch, W., & Schieberle, P. (2004). *Food Chemistry*. Berlin Heidelberg: Springer-Verlag.

Bito, T., Teng, F., & Watanabe, F. (2017). Bioactive compounds of edible purple laver Porphyra sp. (Nori). *Journal of Agricultural and Food Chemistry, 65*(49), 10685-10692.

Blouin, N., Calder, B. L., Perkins, B., & Brawley, S. H. (2006). Sensory and fatty acid analyses of two Atlantic species of Porphyra (Rhodophyta). *Journal of Applied Phycology, 18*(1), 79.

Brown, E. M., Allsopp, P. J., Magee, P. J., Gill, C. I., Nitecki, S., Strain, C. R., & McSorley, E. M. (2014). Seaweed and human health. *Nutrition Reviews*, *72*(3), 205-216.

Cabrita, A. R., Maia, M. R., Oliveira, H. M., Sousa-Pinto, I., Almeida, A. A., Pinto, E. & Fonseca, A. J. (2016). Tracing seaweeds as mineral sources for farm-animals. *Journal of Applied Phycology*, 28, 3135-3150.

Calder, P. C., & Grimble, R. F. (2002). Polyunsaturated fatty acids, inflammation and immunity. *European Journal of Clinical Nutrition, 56*(S3), S14.

Campos, A. M., Matos, J., Afonso, C., Gomes, R., Bandarra, N. M., & Cardoso, C. (2019). Azorean macroalgae (Petalonia binghamiae, Halopteris scoparia and Osmundea pinnatifida) bioprospection: a study of fatty acid profiles and bioactivity. *International Journal of Food Science & Technology, 54*(3), 880-890.

Carvalho, M. L., Santiago, S., & Nunes, M. L. (2005). Assessment of the essential element and heavy metal content of edible fish muscle. *Analytical and Bioanalytical Chemistry*, 382, 426-432.

CEN. (2003). *European Standard EN 14084. Foodstuffs - Determination of trace elements. Determination of lead, cadmium, zinc, copper and iron by atomic absorption spectrometry (AAS) after microwave digestion.* Brussels, Belgium: CEN-European Committee for Standardization.

CEVA. (2014). Edible seaweed and French regulation - Synthesis made by CEVA (31/03/2014),
France. http://www.ceva.fr/eng/INFORMATION/EDIBLEALGAE/Documents-Summaries/Edible-seaweed-and-French-regulation-Synthesis-31-03-2014.

Chan, P. T., & Matanjun, P. (2017). Chemical composition and physicochemical properties of tropical red seaweed, Gracilaria changii. *Food Chemistry, 221*, 302-310.

Chang, H. C., & Wu, L. C. (2008). Texture and quality properties of Chinese fresh egg noodles formulated with green seaweed (*Monostroma nitidum*) powder. *Journal of Food Science*, *73*(8), S398-S404.

Cherry, P., O'Hara, C., Magee, P. J., McSorley, E. M., & Allsopp, P. J. (2019). Risks and benefits of consuming edible seaweeds. *Nutrition Reviews*, *77*(5), 307-329.

Cofrades, S., M. Serdaroğlu, and F. Jiménez-Colmenero. (2013). Design of healthier foods and beverages containing whole algae. In Functional ingredients from algae for foods and nutraceuticals, ed. H. Dominguez, 609–33. Burlington: Woodhead Publishing Series.

Cornish, M. L., Critchley, A. T., & Mouritsen, O. G. (2015). A role for dietary macroalgae in the amelioration of certain risk factors associated with cardiovascular disease. *Phycologia*, *54*(6), 649-666.

Cornish, M. L., Critchley, A. T., & Mouritsen, O. G. (2017). Consumption of seaweeds and the human brain. *Journal of Applied Phycology*, *29*(5), 2377-2398.

da Costa, C. S. G. (2014). Caracterização química de algas disponíveis comercialmente para consumo humano. Master Thesis. Faculdade de Farmácia, Universidade do Porto, Portugal.

Dawczynski, C., Schubert, R., & Jahreis, G. (2007). Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food Chemistry, 103*(3), 891-899.

Dillehay, T. D., Ramírez, C., Pino, M., Collins, M. B., Rossen, J., & Pino-Navarro, J. D. (2008). Monte Verde: seaweed, food, medicine, and the peopling of South America. *Science*, *320*(5877), 784-786.

EC (European Commission). (2006). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of European Union, 364*(365–324).

EC (European Commission). (2018). Commission Recommendation (EU) 2018/464 of 19 March 2018 on the monitoring of metals and iodine in seaweed, halophytes and products based on seaweed. *Official Journal European Union, 78*(16-18).

EFSA (European Food Safety Authority). (2009). Scientific Opinion, Scientific Opinion on Arsenic in Food, EFSA Panel on Contaminants in the Food Chain, EFSA Journal 7(10):1351, 199 pp.

EFSA (European Food Safety Authority). (2017). Dietary Reference Values for nutrients Summary report. EFSA Supporting Publications, 14(12), e15121E.

EFSA (European Food Safety Authority). (2019). (EFSA Panel on Nutrition, Novel Foods and Food Allergens), Turck D, Castenmiller J, de Henauw S, Hirsch-Ernst KI, Kearney J, Maciuk A, Mangelsdorf I, McArdle HJ, Pelaez C, Pentieva K, Siani A, Thies F, Tsabouri S, Vinceti M, Aggett P, Fairweather-Tait S, Martin A, Przyrembel H, Ciccolallo L, de Sesmaisons-Lecarré A, Valtueña Martinez S, Martino L and Naska A. Scientific opinion on Dietary Reference Values for sodium. *EFSA Journal*, 200 pp. doi:10.2903/j.efsa.20YY.NNNN.

EPA. (2007). Test method 7473: Mercury in solids and solutions by thermal decomposition, amalgamation and atomic absorption spectrometry. SW-846, USA, Environment Protection Agency, 17pp.

FAO (Food and Agriculture Organization). (2003). *Food energy - methods of analysis and conversion factors. FAO Food and Nutrition Paper, 77.* Rome: Food and Agriculture Organisation of the United Nations.

FAO (Food and Agriculture Organization). (2016). The State of World Fisheries and Aquaculture. FAO Fisheries and Aquaculture Department, Rome.

Fleurence, J., Gutbier, G., Mabeau, S., & Leray, C. (1994). Fatty acids from 11 marine macroalgae of the French Brittany coast. *Journal of Applied Phycology, 6*(5-6), 527-532.

Francesconi, K. A. (2010). Arsenicspecies in seafood: origin and human health implications. *Pure and Applied Chemistry*, *82*(2), 373-381.

Fujiwara-Arasaki, T., Mino, N., & Kuroda, M. (1984). The protein value in human nutrition of edible marine algae in Japan. In *Eleventh international seaweed symposium* (pp. 513-516). Springer, Dordrecht.

Ghani, M. A., Hajal, M. E., & Daud, N. M. (2018). Effect of consuming brown rice bars containing hoodia gordonii and kappaphycus alvarezii on glycemic index, appetite and food intake in healthy adults. *Malaysian Applied Biology, 47*(5), 55-62.

Goñi, I., Valdivieso, L., & Gudiel-Urbano, M. (2002). Capacity of edible seaweeds to modify in vitro starch digestibility of wheat bread. *Food/Nahrung, 46*(1), 18-20.

Goñi, I., Valdivieso, L., & Garcia-Alonso, A. (2000). Nori seaweed consumption modifies glycemic response in healthy volunteers. *Nutrition Research*, *20*(10), 1367-1375.

González, J. A. (2019). Recetario de Pescados Olvidados y Emergentes en Gran Canaria. (1ª ed.). Las Palmas de Gran Canaria: Fundación Canaria Parque Científico Tecnológico de la ULPGC. ISBN: 978-84-936998-9-5.

Gupta, S., Abu-Ghannam, N., & Scannell, A. G. (2011). Growth and kinetics of Lactobacillus plantarum in the fermentation of edible Irish brown seaweeds. *Food and Bioproducts Processing*, *89*(4), 346-355.

Hall, A. C., Fairclough, A. C., Mahadevan, K., & Paxman, J. R. (2012). Ascophyllum nodosum enriched bread reduces subsequent energy intake with no effect on post-prandial glucose and cholesterol in healthy, overweight males. A pilot study. *Appetite*, *58*(1), 379-386.

Hoang, M. H., Kim, J. Y., Lee, J. H., You, S., & Lee, S. J. (2015). Antioxidative, hypolipidemic, and anti-inflammatory activities of sulfated polysaccharides from Monostroma nitidum. *Food Science and Biotechnology, 24*(1), 199-205.

Holdt, S. L., & Kraan, S. (2011). Bioactive compounds in seaweed: functional food applications and legislation. Journal of Applied Phycology, 23(3), 543-597.

Huang, M., & Yang, H. (2019). Eucheuma powder as a partial flour replacement and its effect on the properties of sponge cake. *LWT, 110*, 262-268.

Hughes, M. F., Beck, B. D., Chen, Y., Lewis, A. S., & Thomas, D. J. (2011). Arsenic exposure and toxicology: a historical perspective. *Toxicological Sciences*, *123*(2), 305-332.

Huss, H. H. (1995). Quality and quality changes on fresh fish. FAO Fisheries Technical paper- 348, FAO, Rome, 203p.

IARC. (2012). Working Group on the Evaluation of Carcinogenic Risks to Humans. Arsenic, metals, fibres, and dusts. *IARC monographs on the evaluation of carcinogenic risks to humans*, *100*(PT C), 11.

ISO (International Organization for Standardization). (1996). *Determination of total phosphorus content – Spectrometric method, ISO 13730:1996 standard. International standards meat and meat products.* Genève, Switzerland: International Organization for

Standardization.

Jiménez-Escrig, A., & Sánchez-Muniz, F. J. (2000). Dietary fibre from edible seaweeds: chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutrition Research*, *20*(4), 585-598.

Jorhem, L. (2000). Determination of metals in food by atomic absorption spectrometry after dry ashing: NMKL collaborative study. *Journal of AOAC International, 83(5),* 1204-1211.

Jung, S. M., Kang, S. G., Son, J. S., Jeon, J. H., Lee, H. J., & Shin, H. W. (2016). Temporal and spatial variations in the proximate composition, amino acid, and mineral content of Pyropia yezoensis. *Journal of Applied Phycology, 28*(6), 3459-3467.

Kılınç, B., Cirik, S., Turan, G., Tekogul, H., & Koru, E. (2013). Seaweeds for food and industrial applications. In *Food Industry*. IntechOpen.

Kumar, K. S., Ganesan, K., & Rao, P. S. (2015). Seasonal variation in nutritional composition of Kappaphycus alvarezii (Doty) Doty—an edible seaweed. *Journal of Food Science and Technology, 52*(5), 2751-2760.

Lall, S. P. (1995). Macro and trace elements in fish and shellfish, In A, Ruiter (Ed.), Fish and fishery products. Composition, nutritive properties and stability (pp. 187-213). Wallingford: Cab International.

López-López, I., Bastida, S., Ruiz-Capillas, C., Bravo, L., Larrea, M. T., Sánchez-Muniz, F., Cofrades, S., & Jiménez-Colmenero, F. (2009). Composition and antioxidant capacity of low-salt meat emulsion model systems containing edible seaweeds. *Meat Science*, *83*(3), 492-498.

López-López, I., Cofrades, S., Yakan, A., Solas, M. T., & Jiménez-Colmenero, F. (2010). Frozen storage characteristics of low-salt and low-fat beef patties as affected by Wakame addition and replacing pork backfat with olive oil-in-water emulsion. *Food Research International*, *43*(5), 1244-1254.

Lourenço, H. M. G. (2011). Essential and toxic elements in fish products consumed in Portugal. PhD Thesis. Faculdade de Ciências, Universidade de Lisboa, Portugal.

Lourenço, H. M., Afonso, C., Anacleto, P., Martins, M. F., Nunes, M. L., & Lino, A. R. (2012). Elemental composition of four farmed fish produced in Portugal. *International Journal of Food Sciences and Nutrition*, 63 (7), 853-859.

Lüning, K., & Pang, S. (2003). Mass cultivation of seaweeds: current aspects and approaches. *Journal of Applied Phycology*, *15*(2-3), 115-119.

MacArtain, P., Gill, C. I., Brooks, M., Campbell, R., & Rowland, I. R. (2007). Nutritional value of edible seaweeds. *Nutrition Reviews*, *65*(12), 535-543.

Machu, L., Mišurcová, L., Vavra Ambrozova, J., Orsavova, J., Mlcek, J., Sochor, J., & Jurikova, T. (2015). Phenolic content and antioxidant capacity in algal food products. *Molecules, 20*(1), 1118-1133.

McHugh, D. J. (2003). A guide to the seaweed industry FAO Fisheries Technical Paper 441. *Food and Agriculture Organization of the United Nations, Rome*.ISSN 0429–9345.

Mišurcová, L., Machů, L. e Orsavová, J. (2011). Seaweed Minerals as Nutraceuticals. *Advances in Food and Nutrition Research*, 64: 371-390.

Mouritsen, O. G. (2013). The science of seaweeds: marine macroalgae benefit people culturally, industrially, nutritionally, and ecologically. *American Scientist*, *101*(6), 458-466.

Mouritsen, O. G., Dawczynski, C., Duelund, L., Jahreis, G., Vetter, W., & Schröder, M. (2013). On the human consumption of the red seaweed dulse (Palmaria palmata (L.) Weber & Mohr). *Journal of Applied Phycology, 25*(6), 1777-1791.

Nunes, M. L., Batista, I., Bandarra, N. M., Morais, M. G., & Rodrigues, P. O. (2008). Produtos da pesca: valor nutricional e importância para a saúde e bem-estar dos consumidores. Publicações avulsas do IPIMAR, (18), 14-16.

Oehlenschlăger, J. (2002). Identifying heavy metals in fish.*In*: Safety and quality issues in fish processing, H. A. Bremer (Ed.), Woodhead Pblising Limited, Cambridge, pp. 95-113.

Oehlenschlăger, J. (1997). Marine fish – a source for essential elements?!, in *Seafood from Producer to Consumer*, Vol. 38, ed. By Luten JB, Borresen T and Oehlenschlăger J. Elsevier, Amsterdam, pp. 587-607.

Oucif, H., Miranda, J. M., Mehidi, S. A., Abi-Ayad, S. M. E. A., Barros-Velázquez, J., & Aubourg, S. P. (2018). Effectiveness of a combined ethanol–aqueous extract of alga Cystoseira compressa for the quality enhancement of a chilled fatty fish species. *European Food Research and Technology, 244*(2), 291-299.

Paiva, L., Lima, E., Patarra, R. F., Neto, A. I. & Baptista, J. (2014). Edible Azorean macroalgae as source of rich nutrients with impact on human health. *Food Chemistry, 164*, 128-135.

Patarra, R. F., Paiva, L., Neto, A. I., Lima, E., & Baptista, J. (2011). Nutritional value of selected macroalgae. *Journal of Applied Phycology, 23*(2), 205-208.

Pereira, L. (2011). A review of the nutrient composition of selected edible seaweeds. In: Pomin, V.H. (Ed.), Seaweed: Ecology, Nutrient Composition and Medical Uses. Nova Science Publishers, New York, pp. 1–47.

Pereira, L. (2016). Edible Seaweeds of the World. P. 453 Boca Raton,FL, USA: CRC Press, Taylor and Francis Group LLC.

Pérez-Lloréns, J.L., Hernández, I., Vergara, J.J., Brun, F.G., León, Á. (2018). Those Curious and Delicious Seaweeds. A Fascinating Voyage from Biology to Gastronomy. Servicio de Publicaciones de la Universidad de Cádiz, Cádiz.

Peteiro, C., Sánchez, N., & Martínez, B. (2016). Mariculture of the Asian kelp Undaria pinnatifida and the native kelp Saccharina latissima along the Atlantic coast of Southern Europe: An overview. *Algal Research*, 15, 9-23.

Pina-Pérez, M. C., Rivas, A., Martínez, A., & Rodrigo, D. (2017). Antimicrobial potential of macro and microalgae against pathogenic and spoilage microorganisms in food. *Food Chemistry*, *235*, 34-44.

Plessi, M., Bertelli, D., & Monzani, A. (2001). Mercury and selenium content in selected seafood. *Journal of Food Composition and Analysis*, 14, 461-467.

Prabhasankar, P., Ganesan, P., Bhaskar, N., Hirose, A., Stephen, N., Gowda, L. R., ...& Miyashita, K. (2009a). Edible Japanese seaweed, wakame (Undaria pinnatifida) as an ingredient in pasta: Chemical, functional and structural evaluation. *Food Chemistry*, *115*(2), 501-508.

Prabhasankar, P., Ganesan, P., & Bhaskar, N. (2009b). Influence of Indian brown seaweed (Sargassum marginatum) as an ingredient on quality, biofunctional, and microstructure characteristics of pasta. *Food Science and Technology International*, *15*(5), 471-479.

Pramanick, P., Zaman, S., Bera, D., Biswas, S., & Mitra, A. (2017). Nutritional Characteristics of Enteromorpha compressa in Indian Sundarbans. *Journal of Science, Engineering, Health and Management, 1*(2), 45-47.

Pulz, O., & Gross, W. (2004). Valuable products from biotechnology of microalgae. *Applied Microbiology and Biotechnology*, *65*(6), 635-648.

Raimundo, J., Caetano, M., & Vale, C, (2004). Geographical variation and partition of metals in tissues of *Octapus vulgaris* along the Portuguese coast, *Science of the Total Environment*, 325, 71-81.

Riget, F., Johannsen, P. e Asmund, G. (1997). Baseline level and natural variability of elements in three seaweed species from west Greenland. *Marine Pollution Bulletin*, 34(3): 171-176.

Rodrigues, D., Freitas, A. C., Pereira, L., Rocha-Santos, T. A., Vasconcelos, M. W., Roriz, M., Rodríguez-Alcalá, L. M., Gomes, A. M. & Duarte, A. C. (2015). Chemical composition of red, brown and green macroalgae from Buarcos bay in Central West Coast of Portugal. *Food Chemistry*, 183, 197-207.

Rubio, C., Napoleone, G., Luis-González, G., Gutiérrez, A. J., González-Weller, D., Hardisson, A., & Revert, C. (2017). Metals in edible seaweed. Chemosphere, 173, 572-579.

Rupérez, P. (2002). Mineral content of edible marine seaweeds. *Food Chemistry*, *79*(1), 23-26.

Sánchez-Machado, D. I., Lopez-Hernandez, J., Paseiro-Losada, P., & Lopez-Cervantes, J. (2004). Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food Chemistry*, 85, 439-444.

Sasaki, K., Ishihara, K., Oyamada, C., Sato, A., Fukushi, A., Arakane, T., ...& Mitsumoto, M. (2008). Effects of fucoxanthin addition to ground chicken breast meat on lipid and colour stability during chilled storage, before and after cooking. *Asian-Australasian Journal of Animal Sciences*, *21*(7), 1067-1072.

Schmid, M., Guihéneuf, F., & Stengel, D. B. (2014). Fatty acid contents and profiles of 16 macroalgae collected from the Irish Coast at two seasons. *Journal of Applied Phycology, 26*(1), 451-463.

Schmid, M., Kraft, L. G., van der Loos, L. M., Kraft, G. T., Virtue, P., Nichols, P. D., & Hurd, C. L. (2018). Southern Australian seaweeds: A promising resource for omega-3 fatty acids. *Food Chemistry, 265*, 70-77.

Senthil, M. A., Mamatha, B. S., & Mahadevaswamy, M. (2005). Effect of using seaweed (Eucheuma) powder on the quality of fish cutlet. *International Journal of Food Sciences and Nutrition*, *56*(5), 327-335.

Shaw, N. S., & Liu, Y. H. (2000). Bioavailability of iron from purple laver (Porphyra spp.) estimated in a rat hemoglobin regeneration bioassay. *Journal of Agricultural and Food Chemistry, 48*(5), 1734-1737.

Sioen, I., Huybrechts, I., Verbeke, W., Van Camp, J., & De Henauw, S. (2007). n-6 and n-3 PUFA intakes of pre-school children in Flanders, Belgium. *British Journal of Nutrition, 98*(4), 819-825.

Smit, A. J. (2004). Medicinal and pharmaceutical uses of edible algae natural products: A review. *Journal of Applied Phycology*, *16*, 245–262.

Taboada, M. C., Millán, R., & Miguez, M. I. (2013). Nutritional value of the marine algae wakame (Undaria pinnatifida) and nori (Porphyra purpurea) as food supplements. *Journal of Applied Phycology*, *25*(5), 1271-1276.

Taylor, V. F., Li, Z., Sayarath, V., Palys, T. J., Morse, K. R., Scholz-Bright, R. A., & Karagas, M. R. (2017). Distinct arsenic metabolites following seaweed consumption in humans. *Scientific Reports*, 7(1), 3920.

Wang, T., Jónsdóttir, R., Kristinsson, H. G., Thorkelsson, G., Jacobsen, C., Hamaguchi, P. Y., & Ólafsdóttir, G. (2010). Inhibition of haemoglobin-mediated lipid oxidation in washed cod muscle and cod protein isolates by Fucus vesiculosus extract and fractions. *Food Chemistry*, *123*(2), 321-330.

Wang, L., Park, Y. J., Jeon, Y. J., & Ryu, B. (2018). Bioactivities of the edible brown seaweed, Undaria pinnatifida: A review. *Aquaculture*, *495*, 873-880.

Wang, T., & Tang, H. (2017). The physical characteristics of human proteins in different biological functions. *PloS one*, *12*(5), e0176234.

Watanabe, F., Takenaka, S., Kittaka-Katsura, H., Ebara, S., & Miyamoto, E. (2002). Characterization and bioavailability of vitamin B12-compounds from edible algae. *Journal of Nutritional Science and Vitaminology*, *48*(5), 325-331.

Wilk, A., Kalisińska, E., Kosik-Bogacka, D. I., Romanowski, M., Różański, J., Ciechanowski, K., ... & Łanocha-Arendarczyk, N. (2017). Cadmium, lead and mercury concentrations in pathologically altered human kidneys. *Environmental Geochemistry and Health*, *39*(4), 889-899.